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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent application of	: Group Art Unit: Not Yet Assigned
	Donald L. Siegel	:
Appln. No:	Not Yet Assigned	: Examiner: Not Yet Assigned
Filed:	Herewith	:
For:	Rh(D)-BINDING PROTEINS AND	: Attorney Docket
	MAGNETICALLY ACTIVATED	: No. **9596-42U3**
	CELL SORTING METHOD FOR	: (53893-5008-02)
	PRODUCTION THEREOF	:

PRELIMINARY AMENDMENT

Preliminary to the calculation of the filing fee and prior to the examination of the above identified application, please amend the application as follows:

In the Specification:

On page 1, immediately below the "title" please add the amended section submitted herewith.

On pages 7-11, please delete the section of the specification entitled "Brief Description of the Drawings" and substitute in place thereof the amended section submitted herewith.

In the Claims:

Please cancel claims 1-10 and 15-21, without prejudice.

Please amend claim 12 to more properly depend from claim 11, instead of claim 10 as originally filed. A marked up copy of claim 12 and a clean copy of amended claim 12 are submitted herewith on separate sheets.

Please add new claims 22-37, as submitted herewith on separate sheets.

REMARKS

Claims 11-14 and 22-37 are pending in the present application.

This application is a divisional of U.S. Application No. 09/240,274 (parent application). Claims 11-14 herein are the original nonelected claims of Group III designated in the Restriction Requirement dated March 16, 2000 in the parent application.

Claim 12 is amended to more properly depend from claim 11, instead of claim 10 as originally filed. Support for claim 12 is found throughout the specification, but is particularly found in the specification in Figures 1-16, Tables 1-4 (pages 35-42 and 102), and on pages 65-97 and 105-126 of the parent application.

Claims 22-37 are being added in the present application to more clearly set forth that which the Applicant regards as his invention. Support for new claims 22-37 is found in the specification in Figures 1-16, Tables 1-4 (pages 35-42 and 102), and on pages 65-97 and 105-126 of the parent application. Thus, no new matter has been added by way of these newly added claims.

Applicants respectfully request favorable examination of all of the pending claims in the application.

Respectfully submitted,

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MAY 4, 2001 (Date)

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KD/rls

Marked Up Copy of Page 1 of the Specification

-- This application is a divisional of U.S. Application No. 09/240,274, now allowed, filed January 29, 1999, which in turn is a continuation-in-part of prior U.S. Application No. 08/884,045, filed June 27, 1997 (now issued U.S. Patent No. 5,876,925), which in turn claims priority under 35 U.S.C. § 119(e) to provisional U.S. Application No. 60/081,380, filed April 10, 1998 and to provisional U.S. Application No. 60/028,550, filed October 11, 1996.--

Clean Copy of Amended Page 1 of the Specification

-- This application is a divisional of U.S. Application No. 09/240,274, now allowed, filed January 29, 1999, which in turn is a continuation-in-part of prior U.S. Application No. 08/884,045, filed June 27, 1997 (now issued U.S. Patent No. 5,876,925), which in turn claims priority under 35 U.S.C. § 119(e) to provisional U.S. Application No. 60/081,380, filed April 10, 1998 and to provisional U.S. Application No. 60/028,550, filed October 11, 1996.--

Marked Up Copy of Claim 12

12. The isolated DNA of claim [10] 11, having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 70-138 and 182-224.

Clean Copy of Amended Claim 12

12. The isolated DNA of claim 11, having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 70-138 and 182-224.

Newly Added Claims

22. The isolated DNA of claim 12, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 70-96 and 182-196.

23. The isolated DNA of claim 22, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 70-96.

24. The isolated DNA of claim 22, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 182-196.

25. The isolated DNA of claim 12, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 97-138 and 197-224.

26. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 97-113.

27. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 114-130.

28. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 131-138 and 197-206.

29. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 207-224.

30. The isolated DNA of claim 11, wherein said encoded protein has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-27 and 139-153.

31. The isolated DNA of claim 30, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 1-27.

32. The isolated DNA of claim 30, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 139-153.

33. The isolated DNA of claim 11, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 28-69 and 154-181.

34. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 28-44.

35. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 45-61.

36. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 62-69 and 154-163.

37. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 164-181.

Clean Copy of all Pending Claims for the Examiner's Convenience

11. An isolated DNA encoding a protein having an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 1-69 and 139-181.

12. The isolated DNA of claim 11, having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 70-138 and 182-224.

13. The isolated DNA of claim 12, being substantially purified.

14. An isolated DNA encoding a protein obtained by

generating a synthetic DNA library in a virus vector expressing said protein;

adding a magnetic label to cells expressing said antigen-bearing moiety;

incubating virus expressing said protein with said magnetically labeled cells in the presence of an excess of non-labeled cells which do not express said antigen-bearing moiety to form a mixture, wherein said virus binds to said magnetically labeled cells;

isolating virus bound cells from said mixture and obtaining DNA encoding said protein therefrom.

22. The isolated DNA of claim 12, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 70-96 and 182-196.

23. The isolated DNA of claim 22, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 70-96.

24. The isolated DNA of claim 22, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 182-196.

25. The isolated DNA of claim 12, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 97-138 and 197-224.

26. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 97-113.

27. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 114-130.

28. The isolated DNA of claim 25, wherein said DNA has a nucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 131-138 and 197-206.

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30. The isolated DNA of claim 11, wherein said encoded protein has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-27 and 139-153.

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35. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 45-61.

36. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 62-69 and 154-163.

37. The isolated DNA of claim 33, wherein said encoded protein has an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 164-181.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram of a strategy for cell-surface Fab-phage panning using magnetically-activated cell sorting.

Figure 2 is a graph depicting cell-surface biotinylation of human RBCs.

Figure 3 is a series of graphs which validate the antigen-positive, antigen-negative cell separation procedure of the invention.

Figure 4 is an image of a microplate agglutination assay wherein anti-Rh(D) Fab/phage agglutination titer was measured.

Figure 5 is an image of a microplate agglutination assay showing determination of Rh(D) binding epitope for selected anti-Rh(D) Fab/phage clones.

Figure 6 is an image depicting the use of Fab/phage antibodies in a gel card assay.

Figure 7 comprises Figures 7A and 7B. Figure 7A is a dendrogram which depicts the relationship among the anti-Rh(D) heavy chains described herein in Example 2. The 28 unique heavy chain clones are organized by V_H family, V_H germline gene, and VDJ rearrangement. Each heavy chain clone is identified by a numeral preceded by a letter ("B" through "E") which denotes its germline gene. The 28 heavy chains comprised 12 distinct VDJ regions, designated VDJ1 - VDJ12. Clones with identical VDJ joins putatively result from intra-clonal diversity of 12 original B lymphocytes. Figure 7B is an alignment of the CDR3 regions of the anti-Rh(D) heavy chains.

Figure 8 comprises Figures 8A, 8B, and 8C. Figure 8A, comprising Figures 8A and 8A-1 through 8A-4, is an alignment of anti-Rh(D) heavy chains to their nearest germline V, D, and J genes. Also illustrated are the putative intermediate heavy chain sequences (Ca, Cb, Da, Db, Dc). The number of nucleotide differences from a germline V_H is tabulated to the right of each sequence. In general, D segments showed poor homology with known D genes so mutations were not scored in these regions. Replacement mutations are indicated with letters, silent mutations are indicated as "*", identities are indicated as ".", and insertions are indicated as "-". Sequences derived from the 5' V_H primers used in library construction are indicated as

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">". Figure 8A is a map demonstrating how Figures 8A-1 to 8A-4 are arranged. Figure 8B, comprising Figures 8B, 8B-1, and 8B-2 is an alignment of the four VH3 genes utilized by anti-Rh(D) heavy chains. Figure 8B is a map depicting the arrangement of Figures 8B-1 and 8B-2. Figure 8C is a dendrogram which depicts the relationship among human VH3 family germline genes, and illustrate relatedness of VH3-21, VH3-30. VH3-33, and VH3-30.3 and the surprising restriction in V_H gene usage. The VH3-30.5 gene is present in only certain haplotypes and is identical to VH3-30.

Figure 9 is an ontogenic tree of anti-Rh(D) heavy chains constructed using nucleotide alignment data. Circles represent isolated and sequenced clones, and diamonds represent putative intermediates. The number of nucleotide mutations from its germline V_H gene is indicated in parentheses below the clone name. The distance along the horizontal axis represents the degree of mutation (including J segments) within the constraints of the diagram.

Figure 10 comprises Figures 10A and 10B. Figure 10A, comprising Figures 10A and 10A-1 through 10A-4, is an alignment of anti-Rh(D) κ light chains to their nearest germline V and J genes, and indicates predominance of DPK-9 usage from the $V_\kappa I$ family. Nomenclature for clones is similar to that for heavy chains but uses the letters "F" through "I". Figure 10A is a map depicting the arrangement of Figures 10A-1 through 10A-4. Figure 10B, comprising Figures 10B, 10B-1, and 10B-2 is an alignment of the four V_κ genes utilized by anti-Rh(D) light chains. Figure 10B is a map showing the arrangement of Figures 10B-1 and 10B-2. Symbols are the same as those used in Figure 8A.

Figure 11 comprises Figure 11A and 11B. Figure 11A, comprising Figures 11A and 11A-1 through 11A-4, is an alignment of anti-Rh(D) λ light chains to their nearest germline V and J genes. Figure 11A is a map depicting the arrangement of Figures 11A-1 through 11A-4. Figure 11B, comprising Figures 11B, 11B-1, and 11B-2, is an alignment of the 10 V_λ germline genes utilized, and illustrates the use of a diverse set of variable region genes derived from multiple families. However, all of the clones use the identical J_λ gene segment. Figure 11B is a map representing the arrangement of Figures 11B-1 and 11B-2. Nomenclature for the clones is similar to

that for heavy chains but uses the letters "J" through "S". Symbols are the same as those used in Figure 8A.

Figure 12, comprising Figures 12A, 12B, and 12C, is a trio of graphs which depict comparisons of variable region gene family usage for anti-Rh(D)-specific clones and randomly-picked, non-Rh(D)-binding clones from original $\gamma_1\kappa$ and $\gamma_1\lambda$ non-selected libraries. [Lightly-hatched] Upwardly-angled bars reveal heterogeneity in V_H (Figure 12A), V_κ (Figure 12B), and V_λ (Figure 12C) family representation *before* selection for anti-Rh(D) specificity. Numbers above bars represent absolute number of clones in that group.

Figure 13 depicts the results of determinations of the Rh(D) binding epitope of anti-Rh(D) Fab/phage clones. The five different agglutination patterns obtained from screening all of the 53 Fab/phage clones are illustrated. The particular clones shown in Figure 13 are identified by their unique heavy chain/light chain pairings using the nomenclature defined in Figures 7, 10, and 11. For E1/M3, reactivity with additional Rh(D) variant cells is required to distinguish its specificity for epD3 from that for epD9. Inclusion of the category IVb cell permits the identification of a new epitope designated "epDX".

Figure 14, comprising Figures 14A and 14B, is matrix illustrating the genetic composition and epitope specificity of anti-Rh(D) antibodies. The horizontal axis represents the unique γ_1 heavy chains and the vertical axis represents the unique λ (Figure 14A) and κ (Figure 14B) light chains (based on amino acid sequence). A shaded pattern at the intersection of a heavy chain/light chain pair indicates the Rh(D) epitope specificity observed for that Fab/phage antibody. A few clones gave mixed patterns of reactivity as described herein. Although heavy chains D1, D15, D16, and D17 differ in nucleotide sequence, these chains have an identical amino acid sequence and thus comprise a single column. Similarly, heavy chains C5 and C8 and λ light chains K1 and K2 encode the same proteins. The pairings of these 28 heavy and 41 light chain nucleotide gene segments, which produced 53 unique Fab transcripts, encoded 43 different Fab proteins, as indicated in the matrix.

Figure 15, comprising Figures 15A, 15B, and 15C, depicts the results of inhibition studies performed using recombinant anti-Rh(D) antibodies. The figures

show results of representative experiments demonstrating the mutual inhibition of antibodies directed at two different Rh(D) epitopes (in this example, epD3 and epD6/7, Figures 15A and 15C), but not between an Rh(D) antibody and an unrelated recombinant anti-RBC antibody (an anti-blood group B antibody, Figure 15B). In Figure 15A, Rh(D)-positive RBCs were incubated with soluble Fabs only, phage-displayed Fabs only, or combinations of the two, as indicated. In Figure 15B, Rh(D)-positive RBCs that were blood group B were used. After washing, RBCs were resuspended in anti-M13 antibody and assessed for agglutination induced by phage-displayed Fabs. Soluble Fabs were used "full-strength" while Fab/phage preparations were present in limiting amounts to increase the sensitivity of the inhibition assay, as described herein. In Figure 15C, mutual inhibition of epD3 and epD6/7 anti-Rh(D) antibodies was demonstrated with Rh(D)-positive RBCs, $\gamma_1\kappa$ and $\gamma_1\lambda$ soluble Fabs, and light chain isotype-specific antisera (see text for details). In these examples, the anti-epD3 and anti-epD6/7 antibodies were clones E1/M3 and D5/I3, respectively. The anti-blood group B antibody was isolated from an IgG phage display library made from the splenic B cells of a blood group O donor.

Figure 16, comprising Figures 16A, 16B, and 16C, depict models for Rh(D) antigen/antibody binding. A conventional model (depicted in Figure 16A) and a model described herein (depicted in Figure 16B) for Rh(D) antigen/antibody binding predict different combining sites and genetic relationships between antibodies. As depicted in Figure 16C, if antibodies directed at different Rh(D) epitopes are clonally related, then the expressed repertoire will differ between Rh(D)-negative and partial Rh(D) individuals.